REVIEW ARTICLE

blood advances

Severe aplastic anemia: allogeneic bone marrow transplantation as first-line treatment

George E. Georges,[1,2] Kris Doney,[1,2] and Rainer Storb[1,2]

[1]Clinical Research Division, Fred Hutchinson Cancer Research Center, Seattle, WA; and [2]Department of Medicine, University of Washington School of Medicine, Seattle, WA

Treatment of severe aplastic anemia has improved significantly over the past 4 decades. This review will summarize the key areas of progress in the use of allogeneic hematopoietic cell transplantation and nontransplant immunosuppressive therapy (IST) for the treatment of aplastic anemia and then summarize the recommendations for first-line treatment. Based on recent data, we argue that guidelines for the initial treatment of patients with newly diagnosed severe aplastic anemia require revision. At the time of diagnosis, before beginning treatment, HLA typing should be done to identify a marrow donor among family members or in the unrelated donor registries, and a marrow transplant should be considered first-line therapy. The priority order of donor source for bone marrow transplantation is: (1) HLA-identical sibling, (2) HLA-matched unrelated donor, and (3) HLA-haploidentical donor if an HLA-matched unrelated donor is not rapidly available. Each of these donor marrow sources may be preferable to nontransplant IST. We make this recommendation because of the long-term persistent risk for disease relapse and secondary myelodysplastic syndrome or acute myeloid leukemia with the use of nontransplant IST for patients with aplastic anemia. In contrast, marrow transplantation is associated with high cure rates of aplastic anemia and a relatively low risk for graft-versus-host disease, with many patients now living for decades without the risk for disease recurrence or the development of clonal disorders. Implementation of this first-line treatment strategy will provide patients with severe aplastic anemia the best chance of long-term disease-free survival.

History

The 2 major current competing treatment strategies for severe aplastic anemia, allogeneic bone marrow transplantation (BMT) and immunosuppressive therapy (IST) with anti-thymocyte globulin (ATG), date back to 1970. That year saw the first successful marrow transplant from an HLA-identical sibling donor.[1] The transplant was carried out based on principles established during years of preclinical experimentation, including the cyclophosphamide (CY)-conditioning regimen, the importance of HLA matching between marrow donor and recipient, and the need for postgrafting immunosuppressive drugs to control graft-versus-host disease (GVHD). The conceptual framework of allogeneic BMT was straightforward: replace the aplastic marrow in the patient with a marrow graft from a healthy donor. This concept was validated by the demonstration of sustained donor-type hematopoiesis in surviving patients. The original concept underlying the use of immunosuppression with ATG was also that of substituting a healthy marrow for a diseased one.[2] ATG was meant to serve as an alternative conditioning regimen for less well-matched marrow grafts and, owing to its long half-life in the circulation, exert control over donor lymphocytes, thereby minimizing the risk for GVHD. However, it soon became apparent that the hematopoietic recovery seen in some patients was derived from the host and not from the graft.[3-5] Moreover, hematopoietic recovery in patients who responded to ATG was not always

Submitted 16 May 2018; accepted 21 July 2018. DOI 10.1182/bloodadvances.2018021162.

complete, which contrasted with the generally complete recovery seen with HLA-matched marrow grafts after CY conditioning. Subsequent prospective randomized trials in ATG-treated patients showed that an HLA-mismatched marrow graft was not required and that ATG on its own sufficed for inducing remission of aplastic anemia in some patients.[6,7] Therefore, the HLA-mismatched marrow infusion after ATG was abandoned.

Early results

Early on, neither of the 2 approaches for treating aplastic anemia was an unqualified success. Although most patients conditioned with CY for HLA-identical marrow grafts showed initial engraftment, a large percentage (36% in the Seattle experience) rejected their grafts, and some remaining patients succumbed to GVHD.[8-10] In the 1970s, long-term survival after allogeneic marrow transplantation was 45%. The problem of graft rejection was predicted from preclinical studies and attributed to sensitization of patients to minor non-HLA antigen disparities with their respective donors through prior blood transfusions.[11,12] Consistent with the preclinical data, graft rejection in untransfused severe aplastic anemia patients was rare, and their long-term survival was ~80%.[13,14]

Mortality from GVHD was a second major problem in the era of postgrafting immunosuppression with methotrexate (MTX) monotherapy.[15] Nevertheless, long-term survivors showed stable polyclonal donor–derived hematopoiesis with normal immune function, and they were leading normal lives.[16,17] Pediatric patients had normal growth and development.[18] Fertility was unimpaired, and patients were able to have normal children.[19] One early problem was the then unknown hepatitis C virus infection, which, based on retrospective analyses, affected 45% of transfused patients.[20] Consequently, a number of transplanted patients eventually developed chronic active hepatitis, liver cirrhosis, and even hepatocellular carcinoma.[21] With the identification of the hepatitis C virus and the development of screening tests, hepatitis C is no longer a significant clinical problem. The incidence of cancers, whether related or unrelated to transplantation, has remained low.[22,23] Retrospective analyses among long-term surviving patients showed an incidence of 4% at 20 years.[24]

The problems seen after IST with ATG were of a different nature. Complete, partial, or only minimal responses were seen in 45% of patients, whereas a majority of patients remained aplastic.[25,26] Moreover, clonal disorders, including paroxysmal nocturnal hemoglobinuria, myelodysplastic syndrome (MDS), and acute myeloid leukemia (AML), were seen in surviving patients. Tichelli et al reported a 26% incidence of MDS/AML, and a larger European study reported a 20% incidence of cancer.[27,28] Similar incidences were reported by other investigators.[29]

How have the problems encountered with the 2 treatment strategies (BMT and IST) for aplastic anemia been addressed and what has the progress been?

Progress in BMT

HLA-matched related grafts

Preclinical studies drove the impressive improvement in survival of marrow-grafted patients, which, at our transplant center, is now at 100%.[30,31] Animal experiments showed that the risk for graft rejection could be minimized by using leukocyte-poor blood products, by irradiating blood products before transfusion, and by introducing a conditioning regimen in which CY alternated every 12 hours with ATG, which resulted in immunosuppressive synergism.[32-34] After these changes were adopted clinically, graft rejection rates declined to <4%.[35] Other preclinical studies focused on GVHD prevention.[36] Synergistic immunosuppression was discovered when MTX was combined with a calcineurin inhibitor, either cyclosporine (CSP) or tacrolimus.[37,38] Subsequent prospective phase 3 randomized clinical trials confirmed superior GVHD prevention with the drug combination compared with either drug alone.[39-42]

Combined CY/ATG conditioning and MTX/calcineurin inhibitor immunosuppression postgrafting have become standard of care. The acute GVHD seen tends to be of only mild to moderate severity, and in almost all patients it responds to first-line therapy with prednisone. As a rule, grade 4 acute GVHD is no longer seen.[24,30,31,35] Also, with ursodiol prophylaxis, liver GVHD has become very rare.[43,44]

Although acute GVHD can be controlled in almost all cases and, thus, is associated with a very low risk for death, chronic GVHD has remained a problem. Cumulative incidences ranging from 0% to 44% have been reported with marrow as the graft source.[45-49] We observed that the incidence of chronic GVHD was 16% among patients in whom marrow grafts were limited to $\leq 2.5 \times 10^8$ nucleated cells per kilogram.[31] Treatment duration for chronic GVHD is long, although that information is generally not provided in published reports. Among our patients, the median treatment duration was 17 months (range, 14 to 69 months). Encouragingly, in all but 1 patient, therapy could be discontinued without recurrence of chronic GVHD. The single patient still on therapy receives once-weekly low-dose MTX. The recommendation to limit the number of infused marrow cells is a promising approach to reduce the risk for chronic GVHD, and this approach awaits confirmation in future trials. Limiting the number of infused marrow cells is in stark contrast to the earlier recommendations made in the 1970s-1980s to infuse large doses of donor marrow cells (and buffy coat) to reduce the risk for graft rejection. It is now clear that the earlier recommendations are no longer valid. Since the 1970s-1980s, the risk for graft rejection has been dramatically reduced by minimizing sensitization to non-HLA antigens through the use of leukocyte-depleted and in vitro irradiation of blood products and by the use of the more immunosuppressive CY/ATG conditioning regimen.[9-15,30-35]

As a consequence of the reduced risks for graft rejection, serious acute GVHD and chronic GVHD, survival of patients given HLA-matched marrow grafts has steadily improved. Most studies now report survival rates ranging from 70% to 90%.[49,50] In our experience, survival of patients younger than 20 years has been 100% for the past 28 years, and a more recent study that included pediatric and adult patients with a median follow-up of 4 years also showed 100% survival.[30,31] For some older patients (>50 years), conditioning with reduced-dose CY plus fludarabine (FLU) has achieved improved survival, with less risk for transplant-related mortality.[51-53] A retrospective combined European Group for Blood and Marrow Transplantation/Center for International Blood and Marrow Transplant Research study of transplants in older patients with aplastic anemia (median age, 58 years; range, 50-77 years) had a median time from diagnosis to transplant of 10 months and included

275 HLA-identical sibling grafts, 224 8/8 HLA-matched unrelated grafts, and 39 7/8 HLA-matched unrelated grafts.[54] The patients' overall 3-year survival was 56%. Two factors adversely affected outcome: grafts from unrelated donors and a performance score $<$ 90%. Although the data support, in principle, the feasibility of allogeneic transplantation in older aplastic anemia patients, the data need to be interpreted with caution given the retrospective nature of the analysis and the possibility of selection bias.

Of note, these improvements have been accomplished with marrow as the stem cell source. Studies using granulocyte colony-stimulating factor (G-CSF)–mobilized peripheral blood stem cells (PBSCs) have consistently shown worse outcomes, in large part due to higher rates and greater severity of acute and chronic GVHD. Thus, marrow remains the transplant product of choice.[49,50,55] Recent published data for HLA-identical sibling transplants are summarized in Table 1.

HLA-matched unrelated marrow transplantation

The outcomes after unrelated marrow grafts for aplastic anemia have steadily improved over the past 3 decades. The most recent publications are summarized in Table 1. A recent analysis of European registry results from 508 unrelated and 940 HLA-identical sibling transplant recipients between 2005 and 2009 reported that the incidence of acute and chronic GVHD was greater for unrelated recipients than for HLA-identical recipients: 25% and 26% vs 13% and 14%, respectively.[50] Although the overall survival after unrelated grafts was less than after HLA-identical sibling transplantation, the difference was not significant. The strongest negative predictor of survival was the use of PBSCs, followed by an interval of diagnosis to transplant $\geq$180 days, patient age $\geq$20 years, no ATG in the conditioning regimen, and positive donor/recipient cytomegalovirus status.[50]

Improvement in the outcome of unrelated donor transplantation has focused on reducing toxicity of the conditioning regimen, with a reduced dose of CY combined with FLU and low-dose total body irradiation (TBI). A recent multicenter trial determined the optimal conditioning regimen for unrelated grafts to include horse/rabbit ATG (30 and 3 mg/kg per day, respectively, on days -4 to -2), CY (50 mg/kg on day -4), FLU (30 mg/m^2 per day on days -5 to -2), and 2- Gy TBI (on day -1).[56] The incidence of acute and chronic GVHD was 23.7% and 22.5%, respectively. With a median follow-up of 17 months, the 1-year overall survival was 97.4%. With longer follow-up, there were 3 deaths (8%): 1 from graft failure and 2 from chronic GVHD. In summary, the combination of ATG, CY (50 mg/kg), FLU (120 mg/m^2), and 2-Gy TBI, followed by a 10/10 HLA-allele matched unrelated marrow graft, appears to be the optimal regimen and graft source.[56] An alternative regimen to consider includes conditioning with CY/FLU/alemtuzumab (Table 1); however, alemtuzumab is associated with a high risk for viral infections.[53,57]

Dufour et al reported on the outcome of 29 children with acquired severe aplastic anemia treated with unrelated grafts as first-line treatment (no IST prior to the transplant). The 2-year overall survival and event-free survival were 96% and 92%, respectively. These results appeared equal to a historical matched group of HLA-identical sibling BMT (group a) but superior to 2 other historical matched groups (b and c). In group a, for HLA-identical sibling marrow transplants (n = 87), the 2-year overall survival and event-free survival were 91% and 87%, respectively; in group b, for patients who received first-line IST with horse ATG and CSP (n = 58), 2-year overall survival and event-free survival were 94% and 40%, respectively; and in group c, for recipients of second-line therapy with unrelated donor transplantation after failure of IST (n = 24), 2-year overall survival and event-free survival were both 74%.[58]

The recent data support the recommendation for first-line treatment with an HLA-matched unrelated marrow transplant for children with acquired severe aplastic anemia who lack an HLA-identical sibling donor.[58-60] Given the improvement in outcomes with FLU-based conditioning regimens with low-dose TBI and CY and ATG or alemtuzumab[53,56] and the increased risk for relapse and late complications after nontransplant IST,[26,28,61] strong consideration should be given to first-line marrow transplantation for patients who are younger than 30 years old with aplastic anemia if a 10/10 HLA-matched unrelated donor is rapidly identified. An urgent unrelated donor search should identify and procure a marrow graft within 4-6 weeks from diagnosis; waiting longer than this will incur an increased risk for infection for the patient. Patients from ethnic minorities who are underrepresented in the unrelated donor registries will probably not have a suitable unrelated donor identified rapidly. Unrelated donors with 9/10 high-resolution HLA matching may also be considered, but there are insufficient data to propose such donors for first-line treatment. Clinical trials prospectively comparing unrelated donor BMT with IST (eg, NCT02845596) are open to enrollment, and the results will help clarify the appropriate first-line treatment. After careful clinical evaluation of an informed patient, it may even be appropriate for some older adults to proceed with first-line unrelated donor hematopoietic cell transplantation (HCT). Although unrelated grafts can be successfully performed after failure of immunosuppression or after evolution to MDS/AML, overall survival is reduced when unrelated transplantation is used as second-line treatment.[58] Our recommendation for considering first-line unrelated donor HCT over IST for patients older than 20 years differs from other published opinions,[62] but the recent evidence for improved long-term survival after unrelated donor HCT supports the broader role of unrelated donor HCT as first-line therapy.

HLA-haploidentical related marrow transplantation

The successful application of posttransplant CY (PT-CY) for HLA-haploidentical grafts was recently reported for aplastic anemia patients. Between July 2011 and August 2016, 13 patients with refractory aplastic anemia received HLA-haploidentical–related marrow grafts.[63] The nonmyeloablative conditioning regimen consisted of rabbit ATG (4.5 mg/kg total dose) from days -9 to -7, CY (14.5 mg/kg CY per day) on days -6 and -5, FLU (30 mg/kg per day) from days -6 to -2, and 2-Gy TBI on day -1. PT-CY (50 mg/kg per day, intravenously) on days $+3$ and $+4$, mycophenolate mofetil on days $+5$ through $+35$, and tacrolimus on day $+5$ through 1 year were administered for GVHD prophylaxis. The median age of the patients was 30 years (range, 11-69 years). All patients had sustained engraftment, 2 patients had mild acute and chronic GVHD that responded to treatment, and each patient surviving for $>$12 months was off immunosuppression. With a median follow-up of 21 months (range, 3 to 64 months), all patients were alive. Other groups have reported similar results with PT-CY and HLA-haploidentical marrow transplants, albeit with equally small numbers of patients.[64]

The use of PT-CY to achieve in vivo T-cell depletion has simplified the clinical use of HLA-haploidentical grafts and has achieved very

Table 1. Select recent reports of HLA-matched sibling and unrelated donor hematopoietic cell transplantation for severe aplastic anemia

Reference (year)	Year of transplant	Patients, n	Age range (median), y	Conditioning program	Hematopoietic source (n)	Prevention of GVHD	Graft rejection/ failure, %	GVHD, % Acute	GVHD, % Chronic	Survival, %	Follow-up, range (median), y
HLA-identical sibling donor transplantation											
30 (2012)	1971-1984 1981-1988 1989-2010	98 19 31	1.8-19 (12.8)	CY CY CY+ATG	BM	MTX CSP+MTX CSP+MTX	22 32 7	21 11 39	21 21 10	66 95 100	11-37.2 (31.1) 15.2-27.1 (23) 0.3-21.5 (6.1)
49 (2012)	1999-2009	1886	1-68 (18) 1-69 (24)	CY+ATG (41%) and various	BM (1163) PBSCs (723)	CSP+MTX (41%) or other	9 10	11 17	11 22	Age ≤20 y: 90 Age >20 y: 74 Age ≤20 y: 76 Age >20 y: 64	(2.1) (2.0)
52 (2009)	1998-2007	30	31-66 (46)	FLU+CY+ATG	BM (20) PBSCs (10)	CSP+MTX or other	3	10	13	77	1.1-6.8 (4.1)
82 (2016)	1999-2014	BMT: 1732 IST: 802	0-20 21-40 >40	BMT various IST various	Compare BMT vs IST	Various for BMT	N/A	N/A	N/A	Age 0-20 y: 86 (BMT); 84 (IST) Age 21-40 y: 76 (BMT); 65 (IST) Age >40 y: 56 (BMT); 58 (IST)	0.2-10
50 (2015)	2005-2009	940	Age >20 y: 50%	Various	BM (566) PBSCs (374)	Various	9	13	14	Low risk: 93 Int. risk: 78 High risk: 67	0.1-9.1 (3.1)
31 (2016)	2006-2015	21	3-52 (15)	CY+ATG	BM $<2.5 \times 10^8$ TNC/kg	CSP+MTX	0	47	16 (24)	100	1-8 (4.0)
Unrelated donor transplantation											
57 (2012)	2000-2010	44	3-19 (8)	FLU+CY+Alem various doses	BM (26) PBSCs (18)	CSP or CSP+MMF or MTX	0	38	12	95	1-6.3 (2.9)
50 (2015)	2005-2009	508	Age >20 y: 53%	Various	BM (264) PBSCs (244)	Various	9	25	26	Low risk: 83 Int. risk: 77 High risk: 64	0.1-9.1 (3.1)
58 (2015)	2005-2014	29 Upfront MUD HCT (no prior IST)	1.7-19.1 (8.4)	FLU+CY+Alem (+2-3-Gy TBI for 1 Ag-MM)	BM (21) PBSCs (8)	CSP or CSP/MMF	4	10	19	96 OS 92 2-y EFS	0.2-8.5 (1.7)
56 (2015)	2006-2013	79	0.5-66 (24)	ATG+2-Gy TBI+ CY-100 (n = 41) vs CY-50 (n = 38)	BM	CSP/MTX	CY-100: 15 CY-50: 12	27 24	32 23	81 at 1 y 97 at 1 y	1.0-4.2 (2.0) 0.3-2.2 (1.4)
53 (2014)	1999-2009	55 URD	1-67 (18)	FLU+CY+Alem	BM (36) PBSCs (19)	Tacrolimus/MMF	9	38	13	88 97 BM 70 PBSCs	0.5-10

Alem, alemtuzumab; BM, bone marrow; CY-50, 50 mg/kg CY; CY-100, 100 mg/kg CY; EFS, event-free survival; HCT, hematopoietic cell transplantation; Int., intermediate; MMF, mycophenolate mofetil; MUD, matched unrelated donor; N/A, not available; OS, overall survival; TBI, total body irradiation; TNC, total nucleated cell; URD, unrelated donor; 1 Ag-MM, 1 HLA-antigen mismatch.

encouraging early results. Again, it appears that bone marrow is the preferred graft source from HLA-haploidentical donors. The results from the Johns Hopkins group challenge the earlier consensus that HLA-haploidentical grafts should be used as second-line therapy for aplastic anemia once immunosuppression has failed and if an HLA-matched unrelated donor is unavailable.[63] Prospective clinical trials evaluating front-line HLA-haploidentical BMT vs IST are needed to settle the issue of the appropriate course of treatment more definitively. Cord blood transplantation is probably inferior to HLA-haploidentical marrow grafts because of the low cell dose infused, the high risk for graft rejection, and delayed engraftment/immune recovery.[65,66]

Nontransplant IST

Progress has also been made with IST for aplastic anemia. The first step forward was to combine ATG with maintenance CSP. A randomized trial conducted in the late 1980s and with long-term follow-up showed ~41% failure-free survival with ATG/CSP compared with only 25% with ATG alone.[25,26] In part, based on that study, current patients receive the combination of ATG and CSP.

In an attempt to improve outcomes, a prospective randomized study from the European Group for Blood and Marrow Transplantation evaluated adding G-CSF to immunosuppression and found no benefit in survival.[67] Moreover, another study identified G-CSF to be a risk factor for the development of MDS/AML with monosomy 7, especially in children.[68] As previously discussed, acquired clonal disorders were identified in several studies, even in the absence of G-CSF, with rates ranging from 13% to 26%.[27-29,69,70]

Rabbit ATG was demonstrated to be inferior to horse ATG as first-line immunosuppressive treatment.[71] A total of 120 consecutive patients with aplastic anemia, ages 2 to 77 years, who were not considered candidates for marrow grafts as first-line treatment, was randomly assigned to horse ATG or rabbit ATG (60 in each group). Horse ATG (40 mg/kg per day for 4 days) was compared with rabbit ATG (3.5 mg/kg per day for 5 days). CSP was given twice daily from day 1 and continued for ≥6 months in both groups. The 2 groups were matched in patient characteristics, and the median follow-up was 28 months. The hematologic response rate at 6 months was 68% with horse ATG and 37% with rabbit ATG ($P < .001$). The incidence of clonal evolution at 3 years was 21% in the horse ATG group and 14% in the rabbit ATG group. The overall survival at 3 years was 96% in the horse ATG group and 76% in the rabbit ATG group when data were censored at the time of marrow transplantation, and it was 94% and 70%, respectively, when not censored.[71]

Eltrombopag (Promacta), an oral thrombopoietin receptor agonist, was used in 43 patients who had remained pancytopenic after initial treatment with ATG + CSP immunosuppression.[72] Although there was an early response rate of 40% at 4 months after starting eltrombopag, only 28% of the patients had a prolonged improvement in blood counts. In this salvage therapy study, response was defined as a prespecified improvement in at least one of the following: platelet, hematocrit, or neutrophil count.[72] However, 19% of treated patients developed cytogenetic abnormalities, including monosomy 7, a poor prognostic finding. More recently, eltrombopag was added to standard immunosuppression (ATG + CSP) as front-line therapy in 92 patents with acquired aplastic anemia.[61] The dose of eltrombopag was evaluated in 3 consecutive cohorts. Cohort 1 received eltrombopag from day 14 to 6 months, cohort 2 received it from day 14 to 3 months, and cohort 3 received it from day 1 to 6 months. The cohorts were analyzed separately. The duration of CSP treatment was increased from 6 to 24 months midway through the trial to reduce disease relapse. The rates of complete response at 6 months were 33% in cohort 1, 26% in cohort 2, and 58% in cohort 3. The overall response rates at 6 months were 80%, 87%, and 94%, respectively. In this front-line therapy study, complete response was defined as recovery of all 3 of the following blood counts at 6 months after the start of treatment: absolute neutrophil count $>1 \times 10^9$/L, hemoglobin >10 g/dL, and platelet count $>100 \times 10^9$/L; overall response included recovery of at least one of these blood counts. The overall survival rate at 2 years was 97% in all cohorts. Marrow transplantation was indicated in 12 patients; 6 patients had not had a response or remained dependent on blood cell transfusions, 3 had a relapse, and 3 had clonal evolution. Clonal cytogenetic evolution occurred in 7 patients (8%); however, the median follow-up was only 2 years. Five patients had loss of chromosome 7, 1 patient had a complex karyotype, and 1 patient had AML. Clonal evolution occurred 3 to 6 months after treatment in 5 patients and at 30 months in 2 other patients. The prolonged duration of IST (24 months) to achieve hematopoietic reconstitution may be significantly longer than after BMT. In summary, the addition of eltrombopag to immunosuppression was associated with higher rates of hematologic response among patients with severe aplastic anemia compared with a historical cohort, but there remained a high incidence of clonal evolution.[61] Eltrombopag is approved by the US Food and Drug Administration for patients with severe aplastic anemia who have had an insufficient response to IST. Recently, it also granted breakthrough therapy designation to eltrombopag for use in combination with nontransplant IST for the treatment of severe aplastic anemia as a first-line therapy.

As described above, it has been recognized that a subset of patients with aplastic anemia treated with immunosuppression develop clonal hematopoiesis and some eventually develop cytogenetic abnormalities that lead to MDS or AML.[27-29] With the development of high-throughput genomic sequencing, the molecular basis of clonal hematopoiesis in aplastic anemia has been comprehensively assessed.[73] Using next-generation sequencing in a study of 439 patients with aplastic anemia, genetic abnormalities were detected in ~50% of patients at ≥6 months after initiation of immunosuppression, most frequently as acquired mutations.[74] One third of these patients had acquired somatic mutations in myeloid cancer candidate genes, including DNMT3A, ASXL1, and BCOR/BCORL1. Severe aplastic anemia patients with ASXL1, DNMT3A, TP53, RUNX1, JAK2, JAK3, and CSMD1 mutations had a significantly lower response to IST and worse overall survival.

A similar study from King's College London found acquired somatic mutations in 19% of aplastic anemia patients, and most involved ASXL1, DNMT3A, and BCOR. Patients with somatic mutations had longer disease duration, shorter telomere lengths, and a 40% risk for subsequent transformation to MDS.[75] In another study, accelerated telomere attrition was seen in aplastic anemia patients with somatic myeloid cancer gene mutations that progressed to MDS or AML with monosomy 7.[76] Yet another study found that mutations in ASXL1 were associated with progression to MDS.[77] Using whole-exome sequencing, one group reported somatic mutations in 73% of aplastic anemia patients involving 51 genes, including PIGA and 6pUPD, and signaling pathways STAT5B and CAMK2G.[78]

In summary, deep-genomic sequencing for commonly mutated MDS/AML genes has revealed a high incidence of mutations that lead to the emergence of clonal evolution and MDS/AML after immunosuppressive treatment of aplastic anemia. However, it is important to note that evidence of clonal hematopoiesis in the setting of bone marrow failure does not reliably predict evolution to leukemia. In one study, only a small minority of aplastic anemia patients with ASXL1 or DNMT3A mutations progressed to AML/MDS during the limited period of follow-up.[79] In the future, genomic sequencing of the common MDS/AML genes may be useful to determine whether patients with poor-prognosis somatic mutations should be referred for allogeneic marrow transplantation either prior to failure of immunosuppression or to prevent the subsequent progression to MDS/AML. Although patients who evolve to MDS/AML after immunosuppression can be successfully treated with marrow grafts, outcomes are inferior to marrow transplantation early after the diagnosis of severe aplastic anemia.[80]

It is also useful to assess for minor paroxysmal nocturnal hemoglobinuria clones and telomere length by flow cytometry of the peripheral blood at the time of aplastic anemia diagnosis. Children with lymphocytes containing short telomeres and the absence of a paroxysmal nocturnal hemoglobinuria clone at diagnosis had a 19% response rate to IST at 6 months.[81] These patients may benefit from first-line BMT, including from matched unrelated and HLA-haploidentical donors.

Taken together, immunosuppressive treatment is effective at ameliorating pancytopenia in a number of patients, but the lack of uniform success, the risk for somatic mutations resulting in evolution of MDS/AML, and the frequent recurrence of aplastic anemia make this treatment strategy a second choice behind marrow transplantation from an HLA-matched family member. Long-term (>10-year) survival with immunosuppression may be no better than 40% to 50%. The recent improvement in outcomes for marrow transplantation using HLA-identical sibling donors, HLA-matched unrelated donors, or HLA-haploidentical related donors strongly supports the decision to proceed directly to transplantation for the majority of patients with newly diagnosed severe aplastic anemia because of the long-term benefit of transplantation over immunosuppression.

Conclusions

Treatment outcomes with allogeneic marrow transplantation and with immunosuppression have improved since 1970. Complete and sustained donor-derived hematopoietic recovery, excellent quality of life, and high rates of survival are now the rule with marrow-transplantation approaches. In contrast, late complications after nontransplant immunosuppression continue to reduce survival. Therefore, faced with the choice of an HLA-identical sibling marrow graft or even an alternative donor graft vs immunosuppression, many patients should be counseled in favor of marrow transplantation as first-line therapy.

Acknowledgments

The work was supported in part by grants from the National Institutes of Health (P01 HL122173 and R01 HL125183 from the National Heart, Lung, and Blood Institute, and P30 CA015704 from the National Cancer Institute).

Authorship

Contribution: G.E.G. conducted the literature review and wrote and edited the manuscript; K.D. edited the manuscript; and R.S. wrote and edited the manuscript.

Conflict-of-interest disclosure: The authors declare no competing financial interests.

ORCID profiles: G.E.G., 0000-0002-0625-0858; K.D., 0000-0002-5525-3748; R.S., 0000-0002-9343-4099.

Correspondence: George E. Georges, Fred Hutchinson Cancer Research Center, 1100 Fairview Ave N, D1-100, Seattle, WA 98109-1024; e-mail: ggeorges@fredhutch.org.

REFERENCES

1. Thomas ED, Storb R, Fefer A, et al. Aplastic anaemia treated by marrow transplantation. *Lancet*. 1972;1(7745):284-289.
2. Mathé G, Amiel JL, Schwarzenberg L, et al. Bone marrow graft in man after conditioning by antilymphocytic serum. *BMJ*. 1970;2(5702):131-136.
3. Mathé G, Amiel JL, Schwarzenberg L, et al. Bone marrow graft in man after conditioning by antilymphocytic serum. *Transplant Proc*. 1971;3(1):325-332.
4. Rogentine GN Jr, Rosenberg EB, Merritt CB, et al. Antilymphocyte globulin as the sole immunosuppressant for bone marrow transplantation in aplastic anemia. A case report. *Transplantation*. 1973;15(5):514-517.
5. Mathé G, Schwarzenberg L, Amiel JL, et al. Bone marrow transplantation after antilymphocyte globulin conditioning–split lymphocyte chimerism. *Transplant Proc*. 1972;4(4):551-557.
6. Doney KC, Weiden PL, Buckner CD, Storb R, Thomas ED. Treatment of severe aplastic anemia using antithymocyte globulin with or without an infusion of HLA haploidentical marrow. *Exp Hematol*. 1981;9(8):829-834.
7. Speck B, Gluckman E, Haak HL, van Rood JJ. Treatment of aplastic anaemia by antilymphocyte globulin with and without allogeneic bone-marrow infusions. *Lancet*. 1977;2(8049):1145-1148.
8. Storb R, Thomas ED, Weiden PL, et al. Aplastic anemia treated by allogeneic bone marrow transplantation: a report on 49 new cases from Seattle. *Blood*. 1976;48(6):817-841.
9. Storb R, Prentice RL, Thomas ED. Marrow transplantation for treatment of aplastic anemia. An analysis of factors associated with graft rejection. *N Engl J Med*. 1977;296(2):61-66.
10. Storb R, Prentice RL, Thomas ED. Treatment of aplastic anemia by marrow transplantation from HLA identical siblings. Prognostic factors associated with graft versus host disease and survival. *J Clin Invest*. 1977;59(4):625-632.

11. Storb R, Epstein RB, Rudolph RH, Thomas ED. The effect of prior transfusion on marrow grafts between histocompatible canine siblings. *J Immunol*. 1970;105(3):627-633.

12. Storb R, Rudolph RH, Graham TC, Thomas ED. The influence of transfusions from unrelated donors upon marrow grafts between histocompatible canine siblings. *J Immunol*. 1971;107(2):409-413.

13. Storb R, Thomas ED, Buckner CD, et al. Marrow transplantation in thirty "untransfused" patients with severe aplastic anemia. *Ann Intern Med*. 1980;92(1):30-36.

14. Anasetti C, Doney KC, Storb R, et al. Marrow transplantation for severe aplastic anemia. Long-term outcome in fifty "untransfused" patients. *Ann Intern Med*. 1986;104(4):461-466.

15. Storb R, Prentice RL, Buckner CD, et al. Graft-versus-host disease and survival in patients with aplastic anemia treated by marrow grafts from HLA-identical siblings. Beneficial effect of a protective environment. *N Engl J Med*. 1983;308(6):302-307.

16. Mathioudakis G, Storb R, McSweeney PA, et al. Polyclonal hematopoiesis with variable telomere shortening in human long-term allogeneic marrow graft recipients. *Blood*. 2000;96(12):3991-3994.

17. Storek J, Joseph A, Espino G, et al. Immunity of patients surviving 20 to 30 years after allogeneic or syngeneic bone marrow transplantation. *Blood*. 2001;98(13):3505-3512.

18. Sanders JE; The Seattle Marrow Transplant Team. The impact of marrow transplant preparative regimens on subsequent growth and development. *Semin Hematol*. 1991;28(3):244-249.

19. Sanders JE, Buckner CD, Amos D, et al. Ovarian function following marrow transplantation for aplastic anemia or leukemia. *J Clin Oncol*. 1988;6(5):813-818.

20. Strasser SI, Myerson D, Spurgeon CL, et al. Hepatitis C virus infection and bone marrow transplantation: a cohort study with 10-year follow-up. *Hepatology*. 1999;29(6):1893-1899.

21. Strasser SI, Sullivan KM, Myerson D, et al. Cirrhosis of the liver in long-term marrow transplant survivors. *Blood*. 1999;93(10):3259-3266.

22. Deeg HJ, Socié G, Schoch G, et al. Malignancies after marrow transplantation for aplastic anemia and fanconi anemia: a joint Seattle and Paris analysis of results in 700 patients. *Blood*. 1996;87(1):386-392.

23. Sanders JE, Woolfrey AE, Carpenter PA, et al. Late effects among pediatric patients followed for nearly 4 decades after transplantation for severe aplastic anemia. *Blood*. 2011;118(5):1421-1428.

24. Kahl C, Leisenring W, Deeg HJ, et al. Cyclophosphamide and antithymocyte globulin as a conditioning regimen for allogeneic marrow transplantation in patients with aplastic anaemia: a long-term follow-up. *Br J Haematol*. 2005;130(5):747-751.

25. Frickhofen N, Kaltwasser JP, Schrezenmeier H, et al; The German Aplastic Anemia Study Group. Treatment of aplastic anemia with antilymphocyte globulin and methylprednisolone with or without cyclosporine. *N Engl J Med*. 1991;324(19):1297-1304.

26. Frickhofen N, Heimpel H, Kaltwasser JP, Schrezenmeier H; German Aplastic Anemia Study Group. Antithymocyte globulin with or without cyclosporin A: 11-year follow-up of a randomized trial comparing treatments of aplastic anemia. *Blood*. 2003;101(4):1236-1242.

27. Tichelli A, Gratwohl A, Würsch A, Nissen C, Speck B. Late haematological complications in severe aplastic anaemia. *Br J Haematol*. 1988;69(3):413-418.

28. Socié G, Henry-Amar M, Bacigalupo A, et al; European Bone Marrow Transplantation-Severe Aplastic Anaemia Working Party. Malignant tumors occurring after treatment of aplastic anemia. *N Engl J Med*. 1993;329(16):1152-1157.

29. Ohara A, Kojima S, Hamajima N, et al. Myelodysplastic syndrome and acute myelogenous leukemia as a late clonal complication in children with acquired aplastic anemia. *Blood*. 1997;90(3):1009-1013.

30. Burroughs LM, Woolfrey AE, Storer BE, et al. Success of allogeneic marrow transplantation for children with severe aplastic anaemia. *Br J Haematol*. 2012;158(1):120-128.

31. Gallo S, Woolfrey AE, Burroughs LM, et al. Marrow grafts from HLA-identical siblings for severe aplastic anemia: does limiting the number of transplanted marrow cells reduce the risk of chronic GvHD? *Bone Marrow Transplant*. 2016;51(12):1573-1578.

32. Bean MA, Storb R, Graham T, et al. Prevention of transfusion-induced sensitization to minor histocompatibility antigens on DLA-identical canine marrow grafts by gamma irradiation of marrow donor blood. *Transplantation*. 1991;52(6):956-960.

33. Storb R, Weiden PL, Deeg HJ, et al. Rejection of marrow from DLA-identical canine littermates given transfusions before grafting: antigens involved are expressed on leukocytes and skin epithelial cells but not on platelets and red blood cells. *Blood*. 1979;54(2):477-484.

34. Storb R, Floersheim GL, Weiden PL, et al. Effect of prior blood transfusions on marrow grafts: abrogation of sensitization by procarbazine and antithymocyte serum. *J Immunol*. 1974;112(4):1508-1516.

35. Storb R, Etzioni R, Anasetti C, et al. Cyclophosphamide combined with antithymocyte globulin in preparation for allogeneic marrow transplants in patients with aplastic anemia. *Blood*. 1994;84(3):941-949.

36. Deeg HJ, Storb R, Appelbaum FR, Kennedy MS, Graham TC, Thomas ED. Combined immunosuppression with cyclosporine and methotrexate in dogs given bone marrow grafts from DLA-haploidentical littermates. *Transplantation*. 1984;37(1):62-65.

37. Deeg HJ, Storb R, Weiden PL, et al. Cyclosporin A and methotrexate in canine marrow transplantation: engraftment, graft-versus-host disease, and induction of intolerance. *Transplantation*. 1982;34(1):30-35.

38. Storb R, Raff RF, Appelbaum FR, et al. FK-506 and methotrexate prevent graft-versus-host disease in dogs given 9.2 Gy total body irradiation and marrow grafts from unrelated dog leukocyte antigen-nonidentical donors. *Transplantation*. 1993;56(4):800-807.

39. Storb R, Deeg HJ, Farewell V, et al. Marrow transplantation for severe aplastic anemia: methotrexate alone compared with a combination of methotrexate and cyclosporine for prevention of acute graft-versus-host disease. *Blood*. 1986;68(1):119-125.

40. Storb R, Deeg HJ, Whitehead J, et al. Methotrexate and cyclosporine compared with cyclosporine alone for prophylaxis of acute graft versus host disease after marrow transplantation for leukemia. *N Engl J Med*. 1986;314(12):729-735.

41. Nash RA, Antin JH, Karanes C, et al. Phase 3 study comparing methotrexate and tacrolimus with methotrexate and cyclosporine for prophylaxis of acute graft-versus-host disease after marrow transplantation from unrelated donors. *Blood*. 2000;96(6):2062-2068.

42. Ratanatharathorn V, Nash RA, Przepiorka D, et al. Phase III study comparing methotrexate and tacrolimus (prograf, FK506) with methotrexate and cyclosporine for graft-versus-host disease prophylaxis after HLA-identical sibling bone marrow transplantation. *Blood*. 1998;92(7):2303-2314.

43. Ruutu T, Juvonen E, Remberger M, et al; Nordic Group for Blood and Marrow Transplantation. Improved survival with ursodeoxycholic acid prophylaxis in allogeneic stem cell transplantation: long-term follow-up of a randomized study. *Biol Blood Marrow Transplant*. 2014;20(1):135-138.

44. Gooley TA, Chien JW, Pergam SA, et al. Reduced mortality after allogeneic hematopoietic-cell transplantation. *N Engl J Med*. 2010;363(22):2091-2101.

45. Storb R, Prentice RL, Sullivan KM, et al. Predictive factors in chronic graft-versus-host disease in patients with aplastic anemia treated by marrow transplantation from HLA-identical siblings. *Ann Intern Med*. 1983;98(4):461-466.

46. Horstmann M, Stockschläder M, Krüger W, et al. Cyclophosphamide/antithymocyte globulin conditioning of patients with severe aplastic anemia for marrow transplantation from HLA-matched siblings: preliminary results. *Ann Hematol*. 1995;71(2):77-81.

47. Atta EH, de Sousa AM, Schirmer MR, Bouzas LF, Nucci M, Abdelhay E. Different outcomes between cyclophosphamide plus horse or rabbit antithymocyte globulin for HLA-identical sibling bone marrow transplant in severe aplastic anemia. *Biol Blood Marrow Transplant*. 2012;18(12): 1876-1882.

48. Locatelli F, Bruno B, Zecca M, et al. Cyclosporin A and short-term methotrexate versus cyclosporin A as graft versus host disease prophylaxis in patients with severe aplastic anemia given allogeneic bone marrow transplantation from an HLA-identical sibling: results of a GITMO/EBMT randomized trial. *Blood*. 2000;96(5):1690-1697.

49. Bacigalupo A, Socié G, Schrezenmeier H, et al; Aplastic Anemia Working Party of the European Group for Blood and Marrow Transplantation (WPSAA-EBMT). Bone marrow versus peripheral blood as the stem cell source for sibling transplants in acquired aplastic anemia: survival advantage for bone marrow in all age groups. *Haematologica*. 2012;97(8):1142-1148.

50. Bacigalupo A, Socié G, Hamladji RM, et al; Aplastic Anemia Working Party of the European Group for Blood Marrow Transplantation. Current outcome of HLA identical sibling versus unrelated donor transplants in severe aplastic anemia: an EBMT analysis. *Haematologica*. 2015;100(5):696-702.

51. Sangiolo D, Storb R, Deeg HJ, et al. Outcome of allogeneic hematopoietic cell transplantation from HLA-identical siblings for severe aplastic anemia in patients over 40 years of age. *Biol Blood Marrow Transplant*. 2010;16(10):1411-1418.

52. Maury S, Bacigalupo A, Anderlini P, et al; Severe Aplastic Anemia Working Party, European Group for Blood and Marrow Transplantation (EBMT-SAAWP). Improved outcome of patients older than 30 years receiving HLA-identical sibling hematopoietic stem cell transplantation for severe acquired aplastic anemia using fludarabine-based conditioning: a comparison with conventional conditioning regimen. *Haematologica*. 2009;94(9):1312-1315.

53. Marsh JC, Pearce RM, Koh MB, et al; British Society for Blood and Marrow Transplantation, Clinical Trials Committee. Retrospective study of alemtuzumab vs ATG-based conditioning without irradiation for unrelated and matched sibling donor transplants in acquired severe aplastic anemia: a study from the British Society for Blood and Marrow Transplantation. *Bone Marrow Transplant*. 2014;49(1):42-48.

54. Rice C, Eikema D, Marsh J, et al. Allogeneic hematopoietic stem cell transplantation in older patients aged 50 years or older with severe aplastic anaemia: Results from the European Society for Blood and Marrow Transplant and the Center for International Blood and Marrow Transplant Research. [abstract] *Blood*. 2017;130(suppl 1). Abstract 2446.

55. Schrezenmeier H, Passweg JR, Marsh JC, et al. Worse outcome and more chronic GVHD with peripheral blood progenitor cells than bone marrow in HLA-matched sibling donor transplants for young patients with severe acquired aplastic anemia. *Blood*. 2007;110(4):1397-1400.

56. Anderlini P, Wu J, Gersten I, et al. Cyclophosphamide conditioning in patients with severe aplastic anaemia given unrelated marrow transplantation: a phase 1-2 dose de-escalation study. *Lancet Haematol*. 2015;2(9):e367-e375.

57. Samarasinghe S, Steward C, Hiwarkar P, et al. Excellent outcome of matched unrelated donor transplantation in paediatric aplastic anaemia following failure with immunosuppressive therapy: a United Kingdom multicentre retrospective experience. *Br J Haematol*. 2012;157(3):339-346.

58. Dufour C, Veys P, Carraro E, et al. Similar outcome of upfront-unrelated and matched sibling stem cell transplantation in idiopathic paediatric aplastic anaemia. A study on behalf of the UK Paediatric BMT Working Party, Paediatric Diseases Working Party and Severe Aplastic Anaemia Working Party of EBMT. *Br J Haematol*. 2015;171(4):585-594.

59. Mackarel J, Iatan M, Kumar L, Storey L, O'Marcaigh A, Smith O. In support of upfront stem cell transplantation as first-line therapy for paediatric patients with idiopathic severe aplastic anaemia who lack a sibling donor. *Br J Haematol*. 2017;177(5):806-808.

60. Mortensen BK, Jacobsen N, Heilmann C, Sengeløv H. Allogeneic hematopoietic cell transplantation for severe aplastic anemia: similar long-term overall survival after transplantation with related donors compared to unrelated donors. *Bone Marrow Transplant*. 2016;51(2):288-290.

61. Townsley DM, Scheinberg P, Winkler T, et al. Eltrombopag added to standard immunosuppression for aplastic anemia. *N Engl J Med*. 2017;376(16): 1540-1550.

62. Bacigalupo A. How I treat acquired aplastic anemia. *Blood*. 2017;129(11):1428-1436.

63. DeZern AE, Zahurak M, Symons H, Cooke K, Jones RJ, Brodsky RA. Alternative donor transplantation with high-dose post-transplantation cyclophosphamide for refractory severe aplastic anemia. *Biol Blood Marrow Transplant*. 2017;23(3):498-504.

64. Clay J, Kulasekararaj AG, Potter V, et al. Nonmyeloablative peripheral blood haploidentical stem cell transplantation for refractory severe aplastic anemia. *Biol Blood Marrow Transplant*. 2014;20(11):1711-1716.

65. Pagliuca S, Peffault de Latour R, Volt F, et al. Long-term outcomes of cord blood transplantation from an HLA-identical sibling for patients with bone marrow failure syndromes: a report from Eurocord, Cord Blood Committee and Severe Aplastic Anemia Working Party of the European Society for Blood

and Marrow Transplantation. *Biol Blood Marrow Transplant*. 2017;23(11):1939-1948.

66. Kudo K, Muramatsu H, Narita A, et al. Unrelated cord blood transplantation in aplastic anemia: is anti-thymocyte globulin indispensable for conditioning? *Bone Marrow Transplant*. 2017;52(12):1659-1661.
67. Gluckman E, Rokicka-Milewska R, Hann I, et al; European Group for Blood and Marrow Transplantation Working Party for Severe Aplastic Anemia. Results and follow-up of a phase III randomized study of recombinant human-granulocyte stimulating factor as support for immunosuppressive therapy in patients with severe aplastic anaemia. *Br J Haematol*. 2002;119(4):1075-1082.
68. Kojima S, Ohara A, Tsuchida M, et al; Japan Childhood Aplastic Anemia Study Group. Risk factors for evolution of acquired aplastic anemia into myelodysplastic syndrome and acute myeloid leukemia after immunosuppressive therapy in children. *Blood*. 2002;100(3):786-790.
69. Gurion R, Gafter-Gvili A, Paul M, et al. Hematopoietic growth factors in aplastic anemia patients treated with immunosuppressive therapy-systematic review and meta-analysis. *Haematologica*. 2009;94(5):712-719.
70. Hama A, Takahashi Y, Muramatsu H, et al. Comparison of long-term outcomes between children with aplastic anemia and refractory cytopenia of childhood who received immunosuppressive therapy with antithymocyte globulin and cyclosporine. *Haematologica*. 2015;100(11):1426-1433.
71. Scheinberg P, Nunez O, Weinstein B, et al. Horse versus rabbit antithymocyte globulin in acquired aplastic anemia. *N Engl J Med*. 2011;365(5):430-438.
72. Desmond R, Townsley DM, Dumitriu B, et al. Eltrombopag restores trilineage hematopoiesis in refractory severe aplastic anemia that can be sustained on discontinuation of drug. *Blood*. 2014;123(12):1818-1825.
73. Ogawa S. Clonal hematopoiesis in acquired aplastic anemia. *Blood*. 2016;128(3):337-347.
74. Yoshizato T, Dumitriu B, Hosokawa K, et al. Somatic mutations and clonal hematopoiesis in aplastic anemia. *N Engl J Med*. 2015;373(1):35-47.
75. Kulasekararaj AG, Jiang J, Smith AE, et al. Somatic mutations identify a subgroup of aplastic anemia patients who progress to myelodysplastic syndrome. *Blood*. 2014;124(17):2698-2704.
76. Dumitriu B, Feng X, Townsley DM, et al. Telomere attrition and candidate gene mutations preceding monosomy 7 in aplastic anemia. *Blood*. 2015;125(4): 706-709.
77. Huang J, Ge M, Lu S, et al. Mutations of ASXL1 and TET2 in aplastic anemia. *Haematologica*. 2015;100(5):e172-e175.
78. Babushok DV, Perdigones N, Perin JC, et al. Emergence of clonal hematopoiesis in the majority of patients with acquired aplastic anemia. *Cancer Genet*. 2015;208(4):115-128.
79. Cooper JN, Young NS. Clonality in context: hematopoietic clones in their marrow environment. *Blood*. 2017;130(22):2363-2372.
80. Kim SY, Le Rademacher J, Antin JH, et al. Myelodysplastic syndrome evolving from aplastic anemia treated with immunosuppressive therapy: efficacy of hematopoietic stem cell transplantation. *Haematologica*. 2014;99(12):1868-1875.
81. Narita A, Muramatsu H, Sekiya Y, et al; Japan Childhood Aplastic Anemia Study Group. Paroxysmal nocturnal hemoglobinuria and telomere length predicts response to immunosuppressive therapy in pediatric aplastic anemia. *Haematologica*. 2015;100(12):1546-1552.
82. Bacigalupo A, Giammarco S, Sica S. Bone marrow transplantation versus immunosuppressive therapy in patients with acquired severe aplastic anemia. *Int J Hematol*. 2016;104(2):168-174.